REMARKS

The above Preliminary Amendment amends the specification to conform to and properly reference the sequences in the Sequence Listing. The attached computer readable form of the Sequence Listing is in ASCII format.

STATEMENT UNDER 37 C.F.R. 1.821(e)-(g)

Applicant's representative submits the following Statement under 37 C.F.R. 1.821(e)-(g):

Applicants' undersigned representative hereby states that the content of the Sequence Listing of the above-captioned patent application, and the computer readable copy filed herewith on a computer disk are believed to be the same. Applicant's representative further states that the Sequence Listing adds no new matter to the application.

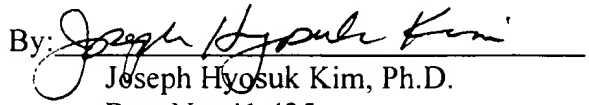
Prompt and favorable consideration on the merits are respectfully requested.

The Commissioner is authorized to charge Squire, Sanders & Dempsey's Deposit Account No. **07-1853** for any fees required under 37 CFR §§ 1.16 and 1.17 that are not covered, in whole or in part, by a check enclosed herewith and to credit any overpayment to said Deposit Account No. **07-1853**.

Respectfully submitted,

SQUIRE, SANDERS & DEMPSEY L.L.P.

Dated: January 9, 2002

By: 
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VERSION MARKED TO SHOW CHANGES MADE

In the Specification

At page 28, please change the following paragraph:

In the DNA construction according to the invention, also any signal sequence permitting the proper exportation of the insulin precursor may be utilized. Preferably, the signal sequence MF α of *S. cerevisiae* that is a peptide comprised of 13 amino acid residues may be employed. The MF α signal sequence has a protease site determined by the sequence of amino acids Lys-Arg-Glu-Ala (SEQ ID NO:26). During the cloning process in pPIC9 of a human insulin precursor gene, this gene may be preferably inserted into the site Xho I that eliminates the –Glu-Ala- residues, whereby the starting insert of the human insulin gene is maintained immediately after the proteases removal site (Figure 1). The cloning in site Xho I permits to obtain a precursor released into the culture medium without remaining amino acids belonging to the signal peptide, thus simplifying the steps of purification of the human insulin.

Primers:

[SEQ ID: N° 1] SEQ ID NO:1: 5′-TCACACCTGG TGGAAGCTCT CTACCTAGTG TGCGGG -3′

[SEQ ID: N° 2] SEQ ID NO:2: 5′-GGTCTTGGGT GTGTAGAAGA AGCCTCGTTC CCCGCACACT AGGTA-3′

[SEQ ID: N° 3] SEQ ID NO:3: 5′- TTTGTGAACC AACACCTGTG CGGCTCACAC CTGGTGGAA -3′

[SEQ ID: N° 4] SEQ ID NO:4: 5′-GCTGGTACAG CATTGTTCCA CAATGCCACG CTTGGTCTTG GGTGT -3′

[SEQ ID: N° 5] SEQ ID NO:5: 5′-CTAGTTGCAG TAGTTCTCCA GCTGGTAGAG GGAGCAGATG CTGGTACAGC AT-3′

Final Product:

[SEQ ID: N° 8] SEQ ID NO:6: 5′- TTTGTGAACC AACACCTGTG CGGCTCACAC CTGGTGGAAG CTCTCTACCT AGTGTGCGGG GAACGAGGCT TCTTCTACAC ACCCAAGACC AAGCGTGGCA TTGTGGAACA ATGCTGTACC AGCATCTGCT CCCTCTACCA GCTGGAGAAC TACTGCAACT AG -3′

(complete insulin precursor)

Replace lines 1-21 at page 42 with the following text.

Construction of an insulin precursor by the polymerase chain reaction (PCR) with the codons more utilized by Pichia pastoris.

Primers:

[SEQ ID: N° 9] SEQ ID NO:7: 5′-ACTTGGTTGA AGCTTTGTAC TTGGTTTGTG GTGAAAGAGG TTTCTTCTAC-3′

[SEQ ID N° 10] SEQ ID NO:8: 5′-AGAAGTACAA CATTGTTCAA CGATACCTCT CTTAGTCTTT GGAGTGTAGA -3′

[SEQ ID:N° 11] SEQ ID NO:9: 5′-ACACTTGTGT GGTTCTCACT TGGTTGAAGC TTT-3′

[SEQ ID:N° 12] SEQ ID NO:10: 5′- TTACTCGAGT TAGTTACAGT AGTTTTCCAA TTGGTACAAA GAACAGATAG AAGTACAACA TTGTTC -3′

[SEQ ID: N° 13] SEQ ID NO:11: 5′-CCGCTCGAGA AGAGATTTGT TAACCAACAC TTGTGT -3′

The obtained product contains the following sequence:

[SEQ ID: N° 14] SEQ ID NO:12:

5′-TTTGTTAACC AACACTTGTG TGGTTCTCAC TTGGTTGAAG CTTTGTACTT GGTTTGTGGT GAAAGAGGTT TCTTCTACAC TCCAAAGACT AAGAGAGGTA

TCGTTGAACA ATGTTGTACT TCTATCTGTT CTTTGTACCA ATTGGAAAAC TACTGTAACT AA-3′

Replace lines 12-20 at page 43 with the following text.

Example 3

Construction of Factor α with preferences codons of Pichia pastoris

By means of this technique the nucleotide sequence corresponding to the leader sequence or signal peptide was cloned.

The employed primers were the following:

SEQ ID NO:13: 5′-CGCGGATCCA AACCATGAGA TTCCCATCTA TCTTCACTGC TGTTTTGTTC GCTGCT -3′--

Replace lines 1-16, at page 44 with the following text.

SEQ ID NO:14: 5′- GTTTTGTTCG CTGCTTCTTC TGCTTTGGCT GCTCCTGTTA ACACTACTAC TGAAGACGAA ACTGCTCA-3′

SEQ ID NO:15: 5′-ACGTCGAAGT CACCTTCCAA GTCAGAGTAA CCGATAACCG CTTCAGCTGG GATTTGAGCA GTTTCGTCTT C -3′

SEQ ID NO:16: 5′-GATGAACAAC AAACCATTAT TAGTAGAGTT AGAGAAAGGC AAAACAGCAA CGTCGAAGTC ACCTTC -3′

SEQ ID NO:17: 5′-CCGCTCGAGA GAAACACCCT CTTCCTTAGC AGCGATAGAA GCGATAGTAG TGTTGATGAA CAACAAACCA TT -3′

The final product has the following sequence:

[SEQ ID N° 20] SEQ ID NO:18

5′-ATGAGATTCC CATCTATCTT CACTGCTGTT TTGTTCGCTG CTTCTTCTGC TTTGGCTGCT CCTGTTAACA CTACTACTGA AGACGAAACT GCTCAAATCC CAGCTGAAGC GGTTATCGGT TACTCTGACT TGGAAGGTGA CTTCGACGTT GCTGTTTTGC CTTTCTCTAA CTCTACTAAT AATGGTTTGT TGTTCATCAA CACTACTATC GCTTCTATCG CTGCTAAGGA AGAGGGTGTT TCTCTCGAGA AGAGAGAGGC TGAAGCA-3′

Replace lines 7-23 at page 45 with the following text.

3- The PCR product [SEQ ID: N° 20] SEQ ID NO:18 was digested with the same restriction enzymes utilized in 1 and was ligated to the fragment obtained in 2.

4- The vector obtained in 3 and the PCR fragment [SEQ ID N° 14] SEQ ID NO:12 was digested with the XhoI, and subsequently they were ligated.

5- The recombinants having the correct orientation of the insulin precursor insert were detected by the HpaI.

EXAMPLE 4

Cloning of insulin precursor gene in pPIC9 yeasts vector

The DNA fragment encoding the insulin precursor was amplified by PCR, employing as a template [SEQ N° 8] SEQ ID NO:6 previously obtained and as primers the following sequences:

[SEQ ID: N° 15.] SEQ ID NO:19: 5′ -[GGGATCCAT] GGGGATCCAT ATGCTCGAGA AAAGATTTGT GAACCAACAC CTGT-3′.

Replace lines 1-2 at page 46 with the following text.

[SEQ ID: N° 16.] SEQ ID NO:20: 5′ -TTAGAATTCC CGGGTCTAGT TGCAGTAGTT CT– 3′.

Replace lines 1-4 at page 49 with the following text.

[SEQ ID: N° 15:] SEQ ID NO:19: 5′ - GGGGATCCAT ATGCTCGAGA AAAGATTTGT GAACCAACAC CTGT-3′

[SEQ ID: N° 17:] SEQ ID NO:21: 5′-TCACTCGAGC GGTCTAGTTG CAGTAGTTCT-3′

Replace lines 16-20 at page 58 with the following text.

His probe that is a fragment 1587 bp of HIS4 gene obtained by digestion of vector Ppic9 with the MscI and the Ins probe that is a fragment 227 bp obtained by PCR employing as a template the plasmid pPIC9IB and the primers corresponding to the sequences [SEQ ID: 15 and 16] SEQ ID NOS:19 and 20.

Replace lines 11-13 at page 62 with the following text.

Sequence of primers:

5′AOX I: 5′ - GACTGGTTCC AATTGACAAG C (SEQ ID NO:25)

3′AOX IN: 5′ - GTCGTGGTTT CTCATAGTAG AGTGGAC (SEQ ID NO:22)

Replace lines 24-25 at page 63 with the following text.

Gap primers:

Gap5': 5′ GGT CAT CAC TGC TCC AT (SEQ ID NO:23)

Replace lines 1 at page 64 with the following text.

Gap3′: 5′ AGC AGC ACC AGT GGA AGA (SEQ ID NO:24)

Replace lines 17-20 at page 64 with the following text.

Insulin precursor primers:

[SEQ ID: N° 15] SEQ ID NO:19: 5′- GGGGATCCAT ATGCTCGAGA AAAGATTTGT GAACCAACAC CTGT

[SEQ ID: N° 15] SEQ ID NO:21: 5′- TCACTCGAGC GGTCTAGTTG CAGTAGTTCT